

**University of Groningen**

## **Validation of the doubly labeled water method in growing precocial birds**

Visser, G.H.; Schekkerman, H

*Published in:*  
Physiological and Biochemical Zoology

*DOI:*  
[10.1086/316713](https://doi.org/10.1086/316713)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
1999

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Visser, G. H., & Schekkerman, H. (1999). Validation of the doubly labeled water method in growing precocial birds. *Physiological and Biochemical Zoology*, 72(6), 740 - 749. <https://doi.org/10.1086/316713>

### **Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### **Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# Validation of the Doubly Labeled Water Method in Growing Precocial Birds: The Importance of Assumptions Concerning Evaporative Water Loss

G. Henk Visser<sup>1,2,\*</sup>

Hans Schekkerman<sup>2,3</sup>

<sup>1</sup>Centre for Isotope Research, Nijenborgh 4, 9747 AG Groningen, The Netherlands; <sup>2</sup>Zoological Laboratory, P.O. Box 14, 9750 AA Haren, The Netherlands; <sup>3</sup>Institute of Forestry and Nature Research, IBN-DLO, P.O. Box 23, 6700 AA Wageningen, The Netherlands

Accepted 9/16/99

## ABSTRACT

The doubly labeled water (DLW) method was validated against respiration gas analysis in growing precocial chicks of the black-tailed godwit (*Limosa limosa*) and the northern lapwing (*Vanellus vanellus*). To calculate the rate of CO<sub>2</sub> production from DLW measurements, Lifson and McClintock's equations (6) and (35) were employed, as well as Speakman's equation (7.17) (all single-pool models). The average errors obtained with the first two equations (+7.2% and -11.6%, respectively) differed significantly from zero but not the error obtained with Speakman's equation (average: -2.9%). The latter error could be reduced by taking a fractional evaporative water loss of 0.13, instead of the value of 0.25 recommended by Speakman. Application of different two-pool models resulted in relative errors of the DLW method of -15.9% or more. After employing the single-pool model with a fractional evaporative water loss value of 0.13, it was found that there was no relationship between the relative growth rate of the chick and the relative error of the DLW method. Recalculation of previously published results on Arctic tern (*Sterna paradisaea*) chicks revealed that the fit of the validation experiment could be considerably improved by employing a single-pool model and assuming a fractional evaporative water loss of 0.20 instead of the value of 0.50 taken originally. After employing the value of 0.20, it was found that there was no relationship between the relative growth rate of the chick and the relative error of the DLW method. This suggests that isotope incorporation into new body substances

does not cause a detectable error. Thus, the DLW method seems to be applicable in young birds growing as fast as 20% d<sup>-1</sup>, after making adjustments for the fractional evaporative water loss. We recommend Speakman's equation (7.17) for general use in growing birds when evaporation is unknown.

## Introduction

The doubly labeled water method (DLW method) has frequently been used to measure the rate of CO<sub>2</sub> production in free-living animals, as well as in humans. In a substantial number of species, the DLW method has been validated for adult animals against classical methods to determine the rate of CO<sub>2</sub> production (Speakman 1997).

During recent years, the DLW method has been used increasingly in growing birds in the field (see, e.g., Williams and Nagy 1985; Williams and Prints 1986; Klaassen et al. 1989; Weathers et al. 1990; Mock et al. 1991; Sullivan and Weathers 1991; Weathers and Sullivan 1991; Gabrielsen et al. 1992; Klaassen 1994; Riedstra et al. 1998). One of the main potential pitfalls of the application of the DLW method in growing birds is that <sup>2</sup>H and <sup>18</sup>O may not leave the body water pool exclusively as water or CO<sub>2</sub> gas but may also disappear from the pool because of incorporation in growing tissues (Williams and Nagy 1985; Williams and Prints 1986; Weathers and Sullivan 1991). This may especially be the case for <sup>2</sup>H, which would result in an overestimation of the <sup>2</sup>H turnover rate as assessed from samples taken from the body water pool and, consequently, lead to an underestimation of the rate of CO<sub>2</sub> production. It was reasoned on the basis of several assumptions that in growing nestling savannah sparrows (*Passerculus sandwichensis*), the DLW method might underestimate the rate of CO<sub>2</sub> production by as much as 25% (Williams and Nagy 1985).

In view of these uncertainties, it is surprising that only two DLW validation studies have been published on growing birds (Klaassen et al. 1989; Gabrielsen et al. 1992). In chicks of the semiprecocial Arctic tern (*Sterna paradisaea*), the DLW method underestimated the "true" rate of CO<sub>2</sub> production by 10.3% on average (Klaassen et al. 1989). To calculate the rate of CO<sub>2</sub> production with the DLW method, Klaassen et al. (1989) used equation (35) of Lifson and McClintock (1966), which corrects for fractionation effects of heavy isotopes by assuming that 50%

\*To whom correspondence should be addressed; e-mail: vissergh@phys.rug.nl.

of the water efflux is lost through evaporative pathways. It was noted that the difference between the DLW and gas analysis estimates increased with the duration of the measurement. In contrast, in the semiprecocial chicks of the black-legged kittiwake (*Rissa tridactyla*), a species taxonomically related to the Arctic tern, DLW estimates calculated by the same equation overestimated the rate of CO<sub>2</sub> production by 28% on the average (Gabrielsen et al. 1992). However, the scatter in the latter study was very large, almost certainly because short measurement intervals (12 h) magnified small differences in isotope enrichments during the experiment to produce large errors (Nagy 1980). In conclusion, there is as yet no clear picture about the applicability of the DLW method in growing birds.

More progress has been made with respect to the application of the DLW method in growing human babies. Models derived for adult humans (Schoeller et al. 1986) also seem to be applicable for growing babies, after making some adjustments for evaporative water loss at low metabolic rates (Fjeld et al. 1989; Roberts 1989). This may lead to the conclusion that differential incorporation of isotopes does not play a major role in growing babies. One may question, however, whether these results are applicable to growing birds because of the low relative growth rates of babies (about 1% d<sup>-1</sup>), compared with levels observed in growing birds (up to 50% d<sup>-1</sup>; Ricklefs 1973).

To evaluate the applicability of the DLW method in growing shorebird chicks, we have conducted a validation study in the northern lapwing (*Vanellus vanellus*) and black-tailed godwit (*Limosa limosa*) at different ages and relative growth rates. After hatching, chicks of these species are self-feeding and receive parental warmth during cold weather (Beintema and Visser 1989a, 1989b). In the field, young chicks of both species achieve maximum growth rates of about 10% d<sup>-1</sup> shortly after hatching. However, during periods of adverse weather, chicks may lose weight because of the limited time the chicks are able to forage and a reduction in foraging success (Beintema and Visser 1989a, 1989b). Values for CO<sub>2</sub> production obtained with respiration gas analysis were compared both with values calculated using equations of Lifson and McClintock (1966) and Speakman (1997) and with a more general model in which the evaporative water loss fraction was not fixed. To evaluate the wider applicability of our findings to growing birds, we recalculated DLW data from the validation study of Klaassen et al. (1989) on Arctic tern chicks, using the model that gave the best fit to our shorebird data.

## Material and Methods

### Animals and Housing

Eggs of both species were collected under permit from the field and transferred to the zoological laboratory. They were incubated in a Comfort incubator set at 37°C. After hatching, chicks were housed in groups of four chicks in wooden holding cages (0.67 × 0.39 × 0.44 m) with a 100-W infrared

heating lamp positioned in one corner. The birds were subjected to a 18L : 6D cycle, with lights on at 0900 h. The birds had ad lib. access to water and food pellets (see Visser and Ricklefs 1993).

### Experimental Procedure

In total, 11 birds were used for the experiments (each animal only once). First, the body mass of the chick was determined (to the nearest gram) with a Sartorius QT 6100 balance. Then, the doubly labeled water mixture (with 62.3% <sup>18</sup>O and 31.9% <sup>2</sup>H) was injected intraperitoneally (IP) after carefully elevating the skin to avoid injection into the air sacs. We preferred IP injections to intramuscular injections because of the small size of leg and pectoral muscles in young chicks. To quantify the dose, the syringe was weighed before and after injection on an analytical balance (Mettler H54) to the nearest 0.1 mg. The dose aimed at ranged between 2 (older chicks) and 5 mg g<sup>-1</sup> (younger chicks). However, in four birds it appeared to be impossible to inject the isotope mixture quantitatively because of leakage of very small quantities of the isotope mixture through the puncture hole of the thin skin. Therefore, in only seven birds could injection be performed quantitatively. After the injection, the bird was placed in a cardboard box without access to water and food. Exactly 1 h after the injection, the bird was reweighed (*M*<sub>i</sub>, g), and the brachial vein was punctured with a small needle. We filled six glass microcapillary tubes each with about 15 μL of blood (initial sample). The microcapillary tubes were flame sealed immediately and stored at 5°C for isotope analysis (see "Isotope Analysis"). Next, the bird was placed in a respiration chamber of appropriate size (enabling the bird to stand and to walk), which was placed in a temperature cabinet employing the same L : D cycle as in the holding cages. Because small chicks are not homeothermic, ambient temperature in the respiration chamber was always adjusted around the chicks' lower critical temperature (Visser 1991; Visser and Ricklefs 1993). During the measurement, water and food were available ad lib., both from the same source as during their stay in the holding cages. Exactly 24 h after taking the initial sample, the bird was taken out of the respiration chamber and immediately weighed again (*M*<sub>f</sub>, g), and six capillary tubes of blood were taken (final sample) as described before. The measurements were performed on two to three birds simultaneously, each bird being housed in a separate chamber. After the measurements, the birds were placed back in their holding cages.

For each bird, the relative growth rate during the measurement (RGR, % d<sup>-1</sup>) was calculated as

$$\text{RGR} = 100 \times (M_f - M_i) / M_i \quad (1)$$

where *M* represents the average body mass (g) during the measurement.

From three birds, blood samples were taken before the injection of the isotopes to determine the average background levels for  $^2\text{H}$  and  $^{18}\text{O}$  during the validation study. We refrained from taking a background sample from each individual because pilot experiments had revealed that such a frequent sampling procedure before the validation experiment could interfere with normal growth. Therefore, a relatively high dose was applied to circumvent the lack of knowledge about the individual-specific background levels (for a discussion on this issue, see Nagy 1980). After the validation study, at ca. 4–5 wk of age, the animals were released in a grassland reserve.

### *Analysis of Respiration Gas*

Respiration gas analysis could be simultaneously performed on three different respiration chambers. Dried compressed air was passed under pressure through each metabolic chamber, and the flow rate for each chamber was measured on the inlet air (Brooks 5850E, with maximum capacities of 60 and 300 L h<sup>-1</sup>). The flow rate changed with age and was adjusted such that the expected CO<sub>2</sub> concentration in the respiration chamber fell between 0.5% and 0.7%. To circumvent a minor alinearity of our mass-flow controllers at very low flow rates (as assessed from our calibrations with a soap foam flow meter; Bubble-O-Meter, La Verne, Calif.), minimum flow rate for each mass-flow controller was always at least 25% of its maximum capacity. The outflow gas of each respiration chamber was dried over a tube filled with molecular sieve (3Å, Merck) and subsequently flushed for 1 min through the CO<sub>2</sub> and O<sub>2</sub> gas analysers (Binos infrared analyser, Leybold Heraeus, and a S-3A/II Oxygen analyser, Applied Electrochemistry, respectively). The reading for that chamber was made during the last 10 s of the 1-min flushing period. Thereafter, the respiration gas of another respiration chamber was led through the gas analysers. For each respiration chamber, the CO<sub>2</sub> and O<sub>2</sub> concentrations of the outflow gas were measured at 6-min intervals. In addition, the CO<sub>2</sub> and O<sub>2</sub> concentrations of the inlet air were measured every 6 min as well. At the start and end of each validation experiment, the gas analysers were calibrated with a certified standard gas mixture (Aktiebolaget Gas Accumulator [AGA], Amsterdam). An interlaboratory comparison was performed with the Agricultural University of Wageningen to verify its CO<sub>2</sub> and O<sub>2</sub> concentrations. The rate of CO<sub>2</sub> production (at conditions of standard temperature, pressure, and dry air) was calculated as the difference between CO<sub>2</sub> concentrations of the outlet and inlet air (Nolet et al. 1992, eq. [3a]), taking into account minor changes in the volumes of outlet and inlet air at respiration quotients <1, times the flow rate (Nolet et al. 1992).

### *Isotope Analysis*

For each sample,  $^2\text{H}/^1\text{H}$  and  $^{18}\text{O}/^{16}\text{O}$  isotope ratios were determined at the Centre for Isotope Research (Speakman et al.

1990). First, the blood in the capillary tube was distilled in a vacuum line. In brief, the sealed capillary tube was placed in a quartz vial connected with a vacuum line. After attainment of vacuum in the system, the vial was closed and the capillary tube was broken mechanically in the vial. Thereafter, the water vapor was cryogenically trapped in a quartz tube using liquid air. We determined  $^{18}\text{O}$  and  $^2\text{H}$  enrichments with the CO<sub>2</sub> equilibration method and the uranium reduction methods, respectively (Speakman 1997). To obtain the carbon dioxide gas for isotope ratio mass spectrometry, 2 mL of CO<sub>2</sub> gas was added quantitatively (based on accurate pressure readings in the system) to the entire distilled water sample to equilibrate for at least 48 h in a thermostatic water bath at 25.0°C (Tamson TC 45). Thereafter, the vial with water and equilibrated CO<sub>2</sub> gas was placed in a dewar with a dry ice–ethanol mixture (to keep the distilled water frozen), and it was connected to the vacuum line to trap the CO<sub>2</sub> gas cryogenically in another quartz vial positioned in liquid air. The remaining water was reduced in a uranium oven at 800°C, and the H<sub>2</sub> gas was cryogenically trapped in a quartz vial with active charcoal, using liquid air. At the end of the transfer process, the pressure of the H<sub>2</sub> gas was assessed to determine the amount of water vapor of the original distilled water sample. The  $^2\text{H}/^1\text{H}$  and  $^{18}\text{O}/^{16}\text{O}$  isotope ratios of the H<sub>2</sub> and CO<sub>2</sub> gas were determined with SIRA 9 isotope ratio mass spectrometer, with a dual inlet for reference and sample gasses.

For each background, initial, or final sample, four capillary tubes were analysed in all cases to determine the  $^2\text{H}$  and  $^{18}\text{O}$  enrichments. During the sample preparation, internal water standards were applied that covered the entire enrichment range of the blood samples. Also, each standard was measured in quadruplicate. In addition, the isotope ratio mass spectrometer was calibrated daily with internal gas standards for  $^2\text{H}$  and  $^{18}\text{O}$  at low and high enrichments. To verify the isotope enrichment of the original DLW mixture, a dilution was made with tap water (with known isotope enrichments), which was analysed in the same batches as the blood samples.

### *Estimates of the Amount of Body Water*

Because of frequent spillages of small parts of the dose through puncture holes in the skin, it was decided that it was not appropriate to use estimates of the amount of body water on the basis of isotope dilution. Therefore, for each bird, the amount of body water (TBW; percentage of the body mass) was estimated indirectly with the following equation, based on carcass analyses of black-tailed godwit and northern lapwing chicks:

$$\text{TBW} = 79.86(1.503 \text{ SE}) - 9.55(3.271 \text{ SE}) \times M/A \quad (2)$$

( $F_{1,6} = 8.56$ ,  $P = 0.026$ ,  $r^2 = 0.59$ ,  $n = 3$  for northern lapwing [body mass range: 15.5–119 g],  $n = 5$  for black-tailed godwit chicks [body mass range: 41.4–202 g]; H. Schekkerman and G.

H. Visser, unpublished data). The variable  $A$  represents the asymptotic body mass for each species (202 and 273 g for lapwing and black-tailed godwit, respectively; H. Schekkerman, unpublished data) and  $M$  the average body mass (g) of the bird during the measurement. This method has also been used in the two other published validation studies (Klaassen et al. 1989; Gabrielsen et al. 1992) and for the determination of energy budgets of free-living chicks (Weathers 1996).

#### Calculation of the $^2\text{H}/^{18}\text{O}$ Dilution Space Ratios

For each bird that was injected without any spillage of the dose, the size of the body water pool ( $N$ , mol) was calculated on the basis of the principle of isotope dilution. Calculations were performed for  $^2\text{H}$  and  $^{18}\text{O}$  dilution by taking into account (1) the quantity ( $Q_d$ , mol) of the dose, (2) the isotope concentration of the dose ( $C_d$ , atom %), (3) the isotope concentration in the bird's body water pool before the administration ( $C_b$ , atom %), and (4) the isotope concentration of the initial blood sample ( $C_i$ , atom %), using the general equation

$$N = Q_d \times (C_d - C_i) / (C_i - C_b). \quad (3)$$

This method has been referred to as the plateau method (Speakman 1997), and for each of the seven animals, one value was yielded on the basis of  $^{18}\text{O}$  dilution ( $N_o$ , mol), and one on the basis of  $^2\text{H}$  dilution ( $N_d$ , mol). For each bird, the ratio of both dilution spaces  $R_{\text{dilspace}}$  (dimensionless) was calculated as  $N_d/N_o$  (Speakman 1997).

For the seven birds that were injected quantitatively, we compared the estimates of the amount of body water based on  $^{18}\text{O}$  dilution (Eq. [3]) with those based on carcass analysis (Eq. [2]). It was found that the difference between the values obtained with carcass analysis and isotope dilution was  $-0.3\%$  (SE = 2.93, range from  $-3.4\%$  to  $4.3\%$ ) on the average. In addition, it was found that there was no relationship between the relative difference between both methods and the body mass of the chick ( $F_{1,6} = 1.32$ ,  $P = 0.30$ ,  $r^2 = 0.21$ ). This suggests that Equation (2) is appropriate to estimate the amount of body water.

#### Fractional Turnover Rates

For each bird, the average initial and final enrichments for each isotope were calculated. Fractional turnover rates for  $^2\text{H}$  and  $^{18}\text{O}$  (henceforth abbreviated as  $k_d$  and  $k_o$ , respectively, units  $\text{d}^{-1}$ ) were calculated following

$$k_d = [\ln(C_{i,^2\text{H}} - C_{b,^2\text{H}}) - \ln(C_{f,^2\text{H}} - C_{b,^2\text{H}})] / t, \quad (4)$$

$$k_o = [\ln(C_{i,^{18}\text{O}} - C_{b,^{18}\text{O}}) - \ln(C_{f,^{18}\text{O}} - C_{b,^{18}\text{O}})] / t, \quad (5)$$

where  $C_{b,^2\text{H}}$  and  $C_{b,^{18}\text{O}}$  represent the average background concentrations for  $^2\text{H}$  and  $^{18}\text{O}$ , respectively (atom %),  $C_{i,^2\text{H}}$  and

$C_{i,^{18}\text{O}}$  the average  $^2\text{H}$  and  $^{18}\text{O}$  concentrations of the initial blood sample, and  $C_{f,^2\text{H}}$  and  $C_{f,^{18}\text{O}}$  the average  $^2\text{H}$  and  $^{18}\text{O}$  concentrations of the final blood sample, and  $t$  the elapsed time interval between taking the initial and final blood sample (d).

#### Water Efflux Rates

First, for each bird, water efflux rates ( $r\text{H}_2\text{O}_{\text{uncorr}}$ ,  $\text{g d}^{-1}$ ) were calculated with Nagy and Costa's (1980) equation (4), which takes into account changes in the size of the body water pool during the measurement but not the fractionation effects of evaporative water loss. Second, water efflux rates ( $r\text{H}_2\text{O}$ ,  $\text{g d}^{-1}$ ) were calculated correcting for isotope fractionation effects caused by evaporative water loss (Speakman 1997, eq. [7.6]):

$$r\text{H}_2\text{O} = r\text{H}_2\text{O}_{\text{uncorr}} / (r_G \times f_1 + 1 - r_G), \quad (6)$$

where  $r_G$  represents the proportion of the water flux lost through evaporative pathways (dimensionless; for our validation experiment taken as 0.13, see "Results"), and  $f_1$  the fractionation factor (taken as 0.94, as recommended by Speakman [1997], p. 107).

#### Rates of $\text{CO}_2$ Production: Single-Pool Models

For each trial, the rate of  $\text{CO}_2$  production ( $r\text{CO}_2$ ,  $\text{L d}^{-1}$ ) was calculated using the following two equations that have been applied in the past to growing birds in the field:

$$r\text{CO}_2 = 22.4 \times [N/2 \times (k_o - k_d)], \quad (7)$$

$$r\text{CO}_2 = 22.4 \times [N/2.08 \times (k_o - k_d) - 0.15Nk_d], \quad (8)$$

where  $N$  stands for the average size of the body water pool during the measurement (mol, derived from Eq. [2]). The factor of 22.4 of the equation was used for the conversion of the volumes from moles to liters. The calculations for  $k_d$  and  $k_o$  are performed with Equations (4) and (5), respectively. Equation (7) was derived by Lifson and McClintock (1966, eq. [6]; henceforth abbreviated as LM-6) and does not take into account the fractionation effects of heavy isotopes. Equation (8), also derived by Lifson and McClintock (1966, eq. [35]; henceforth abbreviated as LM-35), takes fractionation effects into account, assuming that 50% of the water flux rate is lost through evaporative pathways. Recently, Speakman (1997) has reopened the discussion concerning this assumption and argued that a fraction of 25% evaporative water loss might be more appropriate to free-living animals, instead of the original value of 50%. With minor changes in the fractionation constants, this has resulted in the equation

$$r\text{CO}_2 = 22.4 \times [N/2.078 \times (k_o - k_d) - 0.0062Nk_d]. \quad (9)$$

This is a refinement of LM-35 (Lifson and McClintock 1966)

established by Speakman (1997, eq. [7.17]; henceforth abbreviated as SP-7.17).

In an attempt to examine closely the effect of the assumption concerning the fractional evaporative water loss on the fit of the DLW data with those obtained from respiration gas analysis, we also used the more versatile equation

$$r\text{CO}_2 = 22.4 \times [N/2.078 \times (k_o - k_d) - r_G \times 0.0249Nk_d], \quad (10)$$

where  $r_G$  represents the assumed fraction of water flux lost through evaporative pathways (dimensionless; Lifson and McClintock 1966, eq. [34], with the fractionation factors taken from Speakman 1997). For each validation experiment, the rate of  $\text{CO}_2$  production was calculated in the absence of evaporative water loss ( $r_G = 0$ ) and at evaporative water losses of 25%, 50%, and 100% (no defecation) of the water flux rates ( $r_G = 0.25$ , 0.5, and 1, respectively).

#### Rates of $\text{CO}_2$ Production: Two-Pool Models

Two-pool models allow that  $^2\text{H}$  and  $^{18}\text{O}$  dilution spaces differ slightly. Equation (7.43) (Speakman 1997) has been used to calculate the rate of  $\text{CO}_2$  production taking the average  $R_{\text{dilution}}$  value ( $N_d/N_o$ , see "Calculation of the  $^2\text{H}/^{18}\text{O}$  Dilution Space Ratios"; Speakman 1997) obtained from the seven birds.

#### Statistics

For all models employed, the relative error of the  $\text{CO}_2$  production rate (abbreviated as  $r\text{CO}_{2-\text{DLW}}$ , L STPD  $\text{d}^{-1}$ ) was computed for the DLW estimate relative to the estimate obtained with respiration gas analysis (RESP;  $r\text{CO}_{2-\text{RESP}}$ , L STPD  $\text{d}^{-1}$ ), using

$$\text{error} = 100 \times \frac{r\text{CO}_{2-\text{DLW}} - r\text{CO}_{2-\text{RESP}}}{r\text{CO}_{2-\text{RESP}}}. \quad (11)$$

Relative errors of the DLW method were compared for both species using a Student  $t$ -test (two-tailed), and a probability level of 0.05 was taken to determine statistical significance (SPSS/PC+ 4.0; Norušis 1990). In addition,  $t$ -tests were performed to evaluate whether the average error of a given DLW model differed significantly from 0 (Sokal and Rohlf 1995).

## Results

#### Rates of $\text{CO}_2$ Production: Single-Pool Models

In total, 11 validation experiments were performed (six on black-tailed godwit chicks and five on northern lapwing chicks; Table 1). Although all chicks had free access to water and food, some chicks lost mass during the measurements, resulting in

negative relative growth rates (see Eq. [1]) as low as about  $-17\% \text{ d}^{-1}$ . Some other chicks, however, grew as rapidly as about  $15\% \text{ d}^{-1}$ , which is slightly above the maximum growth rates observed for these species in the field. The three models (LM-6, Eq. [7]; LM-35, Eq. [8]; and SP-7.17, Eq. [9]) yielded different estimates of the rate of  $\text{CO}_2$  production (Table 1). For each model, we compared the relative errors of the DLW method obtained for the six black-tailed godwit chicks with those obtained for the five northern lapwing chicks (Eq. [11]; Table 1). The analysis revealed that the relative errors did not differ significantly between the two species (for LM-6:  $t_9 = 0.64$ ,  $P = 0.538$ ; for LM-35:  $t_9 = 1.13$ ,  $P = 0.289$ ; for SP-7.17:  $t_9 = 0.89$ ,  $P = 0.395$ ). For the pooled data set, lowest values of  $\text{CO}_2$  production were obtained with LM-35 (with fractionation effects, assuming that 50% of the water efflux was lost through evaporative pathways), and highest values of  $\text{CO}_2$  production with LM-6 (no fractionation effects). However, the best fit was obtained with SP-7.17 (with fractionation effects, assuming that 25% of the water efflux was lost through evaporative pathways), with an average error of  $-2.9\%$  ( $\text{SD} = 10.09$ ). The error with LM-6 was  $+7.2\%$  ( $\text{SD} = 12.06$ ), and for LM-35, it was  $-11.6\%$  ( $\text{SD} = 10.54$ ). It was found that these average errors were significantly different from 0 for LM-6 ( $t_{10} = 2.37$ ,  $P = 0.04$ ) and LM-35 ( $t_{10} = 3.18$ ,  $P = 0.01$ ) but not for SP-7.17 ( $t_{10} = 0.92$ ,  $P = 0.38$ ). Clearly, the different assumptions concerning fractionation effects had a very strong impact on the overall fit as assessed with the three different equations. Therefore, we tested the effect of the assumption of the fractional evaporative water loss (Eq. [10]) on the relative error of the DLW method (Fig. 1). The relationship between the assumed fractional evaporative water loss ( $r_G$ , dimensionless) and the average relative error of the DLW estimate (error; percentage) can be described with

$$\text{error} = -24.34 \times r_G + 3.15. \quad (12)$$

The best fit was obtained at a fractional evaporative water loss of 0.13, which is lower than the value of 0.25 recommended by Speakman (1997).

For each validation experiment, the rate of carbon dioxide production was recalculated with Equation (10) and a fractional evaporative water loss value of 0.13. This allowed us to evaluate the effect of the chicks' growth rate during the trial (listed in Table 1) on the relative fit of the DLW values (Fig. 2). There was no significant relationship between the relative growth rate of the chick during the measurement and the relative error of the DLW method ( $F_{1,9} = 0.44$ ,  $P = 0.52$ ,  $r^2 = 0.047$ ). This suggests that Equation (10) is applicable to chicks that lose mass, as well as to rapidly growing chicks, after assuming that a fraction of 0.13 of the total water loss occurs via evaporation.

Water efflux rates, calculated from Equation (6), were relatively high and were on average  $111.4\%$  ( $\text{SD} = 47.4\%$ , range  $52\%–187\%$ ) above the levels predicted for captive birds based on Nagy and Peterson's (1988) predictive equation. There was

Table 1: Results of the DLW validation experiments in growing chicks of black-tailed godwit and northern lapwing

Individual <sup>a</sup>	Average Body Mass during the Measurement	Relative Growth Rate during the Measurement (% d <sup>-1</sup> ) <sup>b</sup>	Water Efflux Rate (g d <sup>-1</sup> ) <sup>c</sup>	Rate of CO <sub>2</sub> Production from Respiration Gas Analysis (L d <sup>-1</sup> )	Rate of CO <sub>2</sub> Production Calculated with LM-6 (L d <sup>-1</sup> ) <sup>d</sup>	Rate of CO <sub>2</sub> Production Calculated with LM-35 (L d <sup>-1</sup> ) <sup>e</sup>	Rate of CO <sub>2</sub> Production Calculated with SP-7.17 (L d <sup>-1</sup> ) <sup>f</sup>	Relative Error of LM-6 to Respiration Gas Analysis (%)	Relative Error of LM-35 to Respiration Gas Analysis (%)	Relative Error of SP-7.17 to Respiration Gas Analysis (%)
G1 .....	44.1	14.5	18.3	2.62	2.80	2.26	2.52	6.9	-13.7	-3.8
G2 .....	44.2	5.4	19.4	2.72	3.37	2.85	3.08	23.9	4.8	13.2
G3 .....	72.0	16.1	33.1	5.48	5.55	4.56	5.02	1.3	-16.8	-8.4
G4 .....	81.6	14.3	40.4	5.58	5.49	4.36	4.90	-1.6	-21.9	-12.2
G5 .....	145.7	-10.6	46.1	9.87	12.03	10.93	11.32	21.9	10.7	14.7
G6 .....	162.1	-4.4	58.4	12.00	12.22	10.77	11.36	1.8	-10.3	-5.3
L1 .....	27.7	-13.0	15.6	1.52	1.80	1.52	1.64	18.4	.0	7.9
L2 .....	35.6	-16.9	25.9	2.42	2.35	1.87	2.10	-2.9	-22.7	-13.2
L3 .....	39.9	-1.5	30.5	2.92	2.77	2.10	2.43	-5.1	-28.1	-16.8
L4 .....	75.3	6.1	50.4	5.02	5.45	4.24	4.83	8.6	-15.5	-3.8
L5 .....	97.2	8.8	53.5	7.11	7.54	6.14	6.80	6.0	-13.6	-4.4
Average error .....	...	...	...	...	...	...	...	7.2	-11.6	-2.9
SD .....	...	...	...	...	...	...	...	10.09	12.06	10.54

<sup>a</sup> G denotes black-tailed godwit, and L denotes northern lapwing.<sup>b</sup> Equation (1).<sup>c</sup> Equation (6).<sup>d</sup> Equation (7).<sup>e</sup> Equation (8).<sup>f</sup> Equation (9).

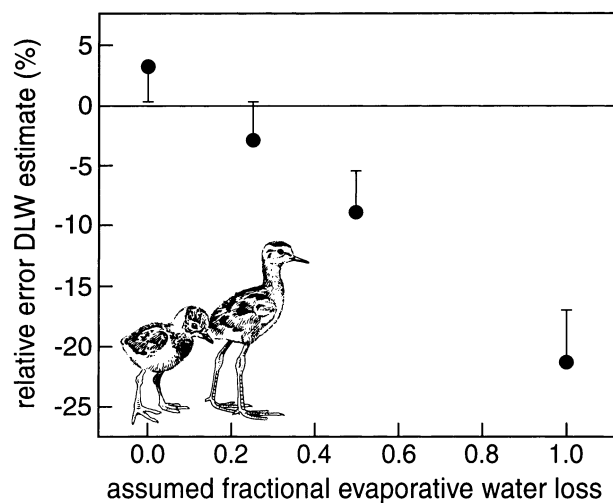


Figure 1. Relationship between the assumed fraction of total water loss that occurs via evaporation to the relative error of the DLW method in growing black-tailed godwit and northern lapwing chicks (Eq. [12]). DLW calculations are based on Equation (10). Error bars indicate 1 SE ( $n = 11$ ).

no significant relationship between the relative growth rate of the chick and its water flux rate relative to the allometrically predicted ( $F_{i,9} = 0.012$ ,  $P = 0.92$ ,  $r^2 = 0.0013$ ).

#### Rates of $\text{CO}_2$ Production: Two-Pool Models

The average  $R_{\text{dilspace}}$  value ( $N_d/N_o$ ) was found to be 1.040 ( $\text{SD} = 0.0187$ ,  $n = 7$ ). There was no relationship between the  $R_{\text{dilspace}}$  value and the initial body mass ( $F_{i,6} = 2.20$ ,  $P = 0.20$ ,  $r^2 = 0.31$ ). After assuming fractional evaporative water loss levels of 0, 0.13, 0.25, and 0.50, it was found that the average errors of the DLW estimate relative to the infrared gas analysis estimate were  $-15.9\%$  ( $\text{SD} = 12.30$ ),  $-19.1\%$  ( $\text{SD} = 12.94$ ),  $-22.2\%$  ( $\text{SD} = 13.55$ ), and  $-28.5\%$  ( $\text{SD} = 14.90$ ), respectively. Clearly, under all these assumptions, the DLW estimates appeared to be too low.

#### Discussion

The results showed that the doubly labeled water method can be used in growing northern lapwing and black-tailed godwit chicks. At a given level of fractional evaporative water loss, the single-pool models gave better fits to the data obtained with respiration gas analysis than the two-pool model. This conforms to the recommendation to apply single-pool models for adult birds smaller than 1 kg (Speakman 1997, p. 161). The fit of the single-pool models was closely related to the assumed fraction of water lost through evaporative pathways. The fractional evaporative water loss value of 0.5, as proposed by Lifson and McClintock (1966), gave larger relative errors (average:

$-11.6\%$ ) compared with an assumed fraction of 0.25, as proposed by Speakman (1997; average relative error of  $-2.9\%$ ), but the best fit was obtained at an assumed fraction of 0.13 (average relative error of 0%).

Unfortunately, we were unable to measure evaporative water loss in these chicks during the measurements. The size of their bills required use of relatively large water trays for the birds. However, Visser (1991) has derived an allometric relationship for rates of evaporative water loss in black-tailed godwit and northern lapwing chicks exposed to temperatures at and below the lower critical temperature. For both species, estimated absolute rates of evaporative water loss ranged from a low 3.4 g in the smallest northern lapwing chick to a high 11.7 g in the largest black-tailed godwit chick. If the estimates of evaporative water losses are compared with the calculated water efflux rates listed in Table 1, the fractional evaporative water losses ranged from a low fraction of 0.15 to a high fraction of 0.24 (average fraction 0.19,  $\text{SD} = 0.026$ ). The average value agrees reasonably well with the assumed fraction of 0.13, which gave the best fit to the DLW data.

#### Recalculation of Klaassen et al.'s (1989) Data on Arctic Tern Chicks

Klaassen et al. (1989) validated the DLW method in growing semiprecocial Arctic tern chicks. When employing LM-35, the authors found that the DLW method yielded an average error of  $-10.3\%$  on the average, that is, very close to the average value of  $-11.6\%$  we found in growing shorebird chicks when

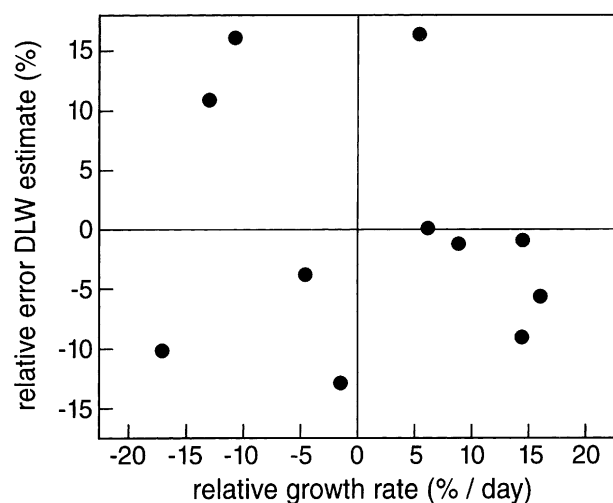


Figure 2. Relationship between the relative growth rate of the chick during the validation measurement and the relative error of the DLW method in growing black-tailed godwit and northern lapwing chicks. DLW calculations are based on Equation (10), assuming that a fraction of 0.13 of the total water loss occurs via evaporation.



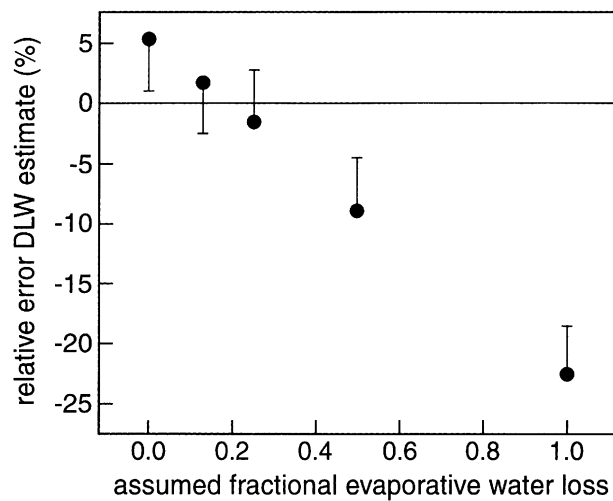


Figure 3. Relationship between the assumed fraction of total water loss that occurs via evaporation to the relative error of the DLW method in growing Arctic tern chicks (Eq. [13]). DLW calculations are based on Equation (10). Error bars indicate 1 SE ( $n = 6$ ).

using the same equation. To evaluate whether the fit of the data set on Arctic tern chicks could be improved, we recalculated the rate of  $\text{CO}_2$  production of Arctic tern chicks in relation to the assumed fractional evaporative water loss employing Equation (10). The relationship between the assumed fractional evaporative water loss ( $r_G$ , dimensionless) and the relative error of the DLW estimate in Arctic tern chicks (error; percentage) can be described with

$$\text{error} = -28.10 \times r_G + 5.52 \quad (13)$$

(Fig. 3). For the Arctic tern, the best fit was found at an assumed fractional evaporative water loss of 0.20, that is, close to the value of 0.13 found in shorebird chicks but much lower than the fraction of 0.5 used in LM-35. Apparently, the DLW method works well in this species also if the level of fractional evaporative water loss is adjusted appropriately. After calculating the rate of  $\text{CO}_2$  production for each bird, employing an evaporative water loss proportion of 0.20, there was no significant relationship between the relative growth rate of the chick during the measurement and the relative error of the DLW method ( $F_{1,4} = 0.30$ ,  $P = 0.61$ ,  $r^2 = 0.06$ ; Fig. 4). Apparently, the DLW method is applicable within the observed range in growth rates, similar to the result obtained in shorebird chicks. Klaassen et al. (1989) identified a tendency for the DLW method to underestimate the rate of  $\text{CO}_2$  production increasingly with the duration of the experiment. This tendency was still present at a fractional evaporative water loss of 0.20: for the first part of the validation measurement for each bird (0–24-h interval), the average error was 6.9% (range: 4.0%–9.1%,  $n = 3$ ), and for the

second part of the validation measurement of each bird (12–36-h interval, which partly overlaps the first part), the average error was  $-6.4\%$  (range:  $-16.8\%$ – $+1.7\%$ ,  $n = 3$ ). Isotope enrichment levels after 36 h, however, were relatively low, which may have resulted in a less accurate estimate of the  $\text{CO}_2$  production during the second part of the experiment (Nagy 1980).

The results obtained on shorebirds and the Arctic tern seem to indicate that the DLW method is accurate in individuals growing as fast as  $20\% \text{ d}^{-1}$  after making some assumptions concerning fractional evaporative water loss. Still, validation studies are urgently needed for small rapidly growing passerine bird species, the chicks of which can achieve growth rates of  $50\% \text{ d}^{-1}$  or more (Ricklefs 1973), and for growing birds having different diets that result in differences in water flux rates. The results obtained so far, however, do not exclude the hypothesis that  $^2\text{H}$  and  $^{18}\text{O}$  are incorporated in growing tissue, but if this occurs, apparently the incorporation rates are not differential. In fact, our correction for fractional evaporative water loss may correct for this process. Analyses of isotope incorporation into tissues, collected after the validation experiment, are needed to address this question in more detail.

Another unresolved issue is the accurate assessment of the values for the fractionation constants for the  $^2\text{H}$  ( $f_1$ , fractionation water vapor to water) and  $^{18}\text{O}$  isotope ( $f_2$ , fractionation water vapor to water, and  $f_3$ , fractionation  $\text{CO}_2$  gas to bicarbonate in water; Lifson and McClintock 1966; Speakman 1997, p. 107). Because these fractionation processes are influenced by temperature, the uncertainty concerning the appropriate fractionation constants is probably larger in growing poikilo-

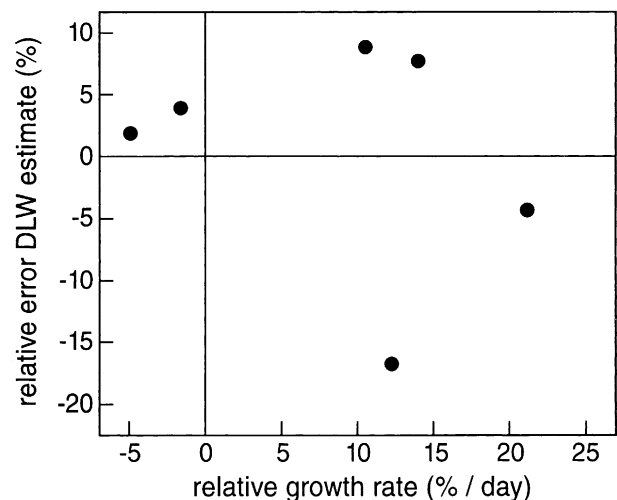


Figure 4. Relationship between the relative growth rate of the chick during the validation measurement and the relative error of the DLW method in growing Arctic tern chicks. DLW calculations are based on Equation (10), assuming that a fraction of 0.20 of the total water loss occurs via evaporation.

thermic chicks than in homeothermic adults. In addition, values may differ among species because of differences in body temperature. Any error in the assumed values will propagate in the final estimates for the rate of CO<sub>2</sub> production (Lifson and McClintock 1966; Nagy 1980; Speakman 1997). Based on the validation experiments in black-tailed godwit, northern lapwing, and Arctic tern, for general use in growing birds, we recommend Speakman's equation (7.17) when fractional evaporative water losses are unknown.

### Acknowledgments

We thank P. Boon, G. Overkamp, and I. Tieleman for the assistance during blood sampling and the measurements of the respiration gases and T. Dijkstra and B. Verstappen-Dumoulin for conducting the isotope analyses. Dr. Heetkamp from the Agricultural University Wageningen was very helpful during the interlaboratory comparison of the certified gas standards. This study was supported by grants from the "Dienst Landelijk Gebied," Netherlands, Ministry of Agriculture, Nature Management, and Fisheries to A. J. Beintema, and National Science Foundation OPP-9696134 to R. E. Ricklefs. M. Klaassen generously made available to us his raw data on the validation study of the Arctic tern. Critical comments from D. M. Bryant and R. E. Ricklefs improved the manuscript.

### Literature Cited

- Beintema A.J. and G.H. Visser. 1989a. The effect of weather on time budgets and development of chicks of meadow birds. *Ardea* 77:181–192.
- . 1989b. Growth parameters in chicks of Charadriiform birds. *Ardea* 77:169–180.
- Fjeld C.R., D.A. Schoeller, and K.H. Brown. 1989. A new model for predicting energy requirements of children during catch-up growth developed using doubly labeled water. *Pediatr Res* 25:503–508.
- Gabrielsen G.W., M. Klaassen, and F. Mehlum. 1992. Energetics of black-legged kittiwake *Rissa tridactyla* chicks. *Ardea* 80: 29–40.
- Klaassen M. 1994. Growth and energetics of tern chicks from temperate and polar environments. *Auk* 111:525–544.
- Klaassen M., C. Bech, D. Masman, and G. Slagsvold. 1989. Growth and energetics of Arctic tern chicks (*Sterna parasidis*). *Auk* 106:240–248.
- Lifson N. and R. McClintock. 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. *J Theor Biol* 12:46–74.
- Mock P.J., M. Khubesian, and D.M. Larcheveque. 1991. Energetics of growth and maturation in sympatric passerines that fledge at different ages. *Auk* 108:34–41.
- Nagy K.A. 1980. CO<sub>2</sub> production in animals: analysis of potential errors in the doubly labelled water method. *Am J Physiol* 7:R466–R473.
- Nagy K.A. and D.P. Costa. 1980. Water flux in animals: analysis of potential errors in the tritiated water method. *Am J Physiol* 7:R454–R465.
- Nagy K.A. and S.C. Peterson. 1988. Scaling of water flux rate in animals. University of California Publications in Zoology 120. University of California Press, Berkeley.
- Norušis, M.J. 1990. SPSS/PC+, Version 4.0, Base Manual for the IBM PC/XT/AT and PS/2. SPSS, Chicago.
- Nolet B.A., P.J. Butler, D. Masman, and A.J. Woakes. 1992. Estimation of daily energy expenditure from heart rate and doubly labeled water in exercising geese. *Physiol Zool* 65: 1188–1216.
- Ricklefs R.E. 1973. Patterns of growth in birds. II. Growth rate and mode of development. *Ibis* 115:177–201.
- Riedstra B., C. Dijkstra, and S. Daan. 1998. Daily energy expenditure in male and female marsh harrier nestlings. *Auk* 115:635–641.
- Roberts S.B. 1989. Use of the doubly labeled water method for measurement of energy expenditure, total body water, water intake, and metabolizable energy intake in humans and small animals. *Can J Physiol Pharmacol* 67:1190–1198.
- Schoeller D.A., C.A. Leitch, and C. Brown. 1986. Doubly labeled water method: in vivo oxygen and hydrogen isotope fractionation. *Am J Physiol* 251:R1137–R1143.
- Sokal R.R. and F.J. Rohlf. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. 3d ed. W. H. Freeman, New York.
- Speakman J.R. 1997. Doubly Labelled Water: Theory and Practice. Chapman & Hall, London.
- Speakman J.R., K.A. Nagy, D. Masman, W.G. Mook, S.D. Po-pitt, G.E. Strathearn, and P.A. Racey. 1990. Interlaboratory comparison of different analytical techniques for the determination of oxygen-18 abundance. *Anal Chem* 62: 703–708.
- Sullivan K.A. and W.W. Weathers. 1991. Brood size and thermal environment influence field metabolism of nestling yellow-eyed juncos. *Auk* 109:112–118.
- Visser G.H. 1991. Development of Metabolism and Temperature Regulation in Precocial Birds: Patterns in Shorebirds (Charadriiformes) and the Domestic Fowl (*Gallus domesticus*). Ph.D. diss. University of Utrecht, Utrecht.
- Visser G.H. and R.E. Ricklefs. 1993. Development of temperature regulation in shorebirds. *Physiol Zool* 66:771–792.
- Weathers W.W. 1996. Energetics of postnatal growth. Pp. 461–496 in C. Carey, ed. *Avian Energetics and Nutritional Ecology*. Chapman & Hall, New York.
- Weathers W.W., W.D. Koenig, and M.T. Stanback. 1990.

- 
- Breeding energetics and thermal ecology of the acorn woodpecker in central coastal California. *Condor* 92:341–359.
- Weathers W.W. and K.A. Sullivan. 1991. Growth and energetics of nestling yellow-eyed juncos. *Condor* 93:138–146.
- Williams J.B. and K. A. Nagy. 1985. Water flux and energetics of nestling savannah sparrows in the field. *Physiol Zool* 58: 515–525.
- Williams J.B. and A. Prints. 1986. Energetics of growth of nestling savannah sparrows: a comparison of doubly labeled water and laboratory estimates. *Condor* 8:74–83.